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Tobacco anionic peroxidase often increases resistance to insects in different dicotyledonous species

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Abstract: Different species and strains of tobacco (*Nicotiana* spp), tomato (*Lycopersicon esculentum*) and sweetgum (*Liquidambar styraciflua*) that had total peroxidase activity enhanced by a few- to over 100-fold through the expression of a tobacco anionic peroxidase gene driven by a cauliflower mosaic promoter were compared with wild-type plants for resistance to relevant insects. Reduced levels of feeding were generally noticed for leaves, stems and fruit, but the age of tissues and insects influenced the response. Enhanced resistance to *Helicoverpa zea* and *Manduca sexta* were noted for tobacco and tomato, and resistance to *Hyphantria cunea* and *Lymantria dispar* were noted for sweetgum. In several cases increased mortality and/or reduced growth rates were noted for the high-peroxidase plants. Although many modes of action are possible, indirect comparisons and gravitational nutritional studies suggest peroxidase-enhanced rates of production of compounds toxic to the insects are the most important.

Keywords: *Lymantria*; *Helicoverpa*; tobacco; tomato; sweetgum; resistance

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1 INTRODUCTION

Peroxidases, in the presence of appropriate cofactors and substrates, have the potential to generate chemicals that are more active against pests than the original substrates. There are indications that peroxidases may be involved in resistance to insects in some plant species.[1] The cloning of a tobacco anionic peroxidase gene, coupled with successful transformation efforts, have made it possible to examine the effect on insects of increased peroxidase activity in different tobaccos (*Nicotiana* spp), tomato (*Lycopersicon esculentum ceraseiforme* Alef) and sweetgum (*Liquidambar styraciflua* L).

2 EXPERIMENTAL METHODS

All plant lines had a chimeric tobacco anionic peroxidase gene introduced via *Agrobacterium*, and driven by a CaMV 35S promoter. Transformation and regeneration of plants has been described previously.[2–4] Stable transformants or their progeny were used in these studies. Peroxidase activity was extracted and analyzed spectrophotometrically using guaiacol as a substrate.[5]

Insects tested with tobacco and tomato included caterpillar pests *Helicoverpa zea* (Boddie) and *Manduca sexta* Joh. A general fruit and vegetable beetle pest, *Carpophilus lugubris*, was also used in some assays. Insects tested with sweetgum included caterpillar pests *Hyphantria cunea* Drury, *Malacosoma americanum* F, and *Lymantria dispar* L. The generalist dried foliage pest *Lasioderma serricorne* F was also tested. The caterpillars *H zea* and *Ostrinia nubilalis* Hübn were also tested with sweetgum.

Two main types of bioassay were used: caged whole plant and excised tissue.[5,6] Relative feeding damage, mortality, and weights of survivors were determined. For sweetgum, some bioassays were set up to determine standard gravimetric nutritional indices.[6]

3 RESULTS

Relative total peroxidase activity is increased by a few- to five-fold for tobacco, up to 10-fold for sweetgum, and over 100-fold for tomato.[1,5,6] About two-fold increased resistance to leaf feeding by first instars of *H zea* was noted for tobacco and tomato transgenic vs wild-type plants, but the opposite trend was noted for transgenic sweetgum.[1,5,6] Third-instar larvae of *H zea* were able to feed on transgenic plants as well as on wild-type ones.[1] Transgenic tobacco and tomato

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leaves have also often been significantly more resistant to first-instar *M sexta* feeding than those from wild-type plants (Dowd PF and Lagrimini LM., unpublished). Intermediate-age leaves of transgenic tobacco and tomato typically show greater reductions in feeding relative to wild-type plants compared to very young, or very old leaves (Dowd PF and Lagrimini LM., unpublished).[5] Basal stems of *Nicotiana sylvestris* are significantly more resistant to feeding by *H zea* (as indicated by significantly higher insect mortality), while terminal stems are not.[1] Transgenic tobacco and tomato stems are also both significantly more resistant to feeding by *C lugubris* than wild-type stems.[1]

Sweetgum expressing tobacco anionic peroxidase showed significantly higher resistance to several species of insect compared to wild-type plants, which ranged from $1.6\times$ (for *O nubilalis*) to $31\times$ (for *H cunea*), but, as indicated previously, was more susceptible to feeding by *H zea*.[6] Although transgenic sweetgum significantly reduced growth rates of *L dispar* by 33%, and caused significantly less consumption relative to wild-type plants (21% reduction), this result was not related to any significant effect on the ability of the insects to digest the leaves.[6] However there was some indication that the transgenic leaves reduced the ability of the insects to convert digested food to biomass,[6] which could also be related to a toxic effect.

4 DISCUSSION

Although transgenic tissues expressing tobacco anionic peroxidase are generally more resistant to feeding by insects, insect age and species, and plant tissue type and age, can influence the degree of resistance noted (if any). The effect on insects feeding on transgenic tobacco and tomato relative to wild-type plants, which have relatively similar secondary metabolite profiles compared to sweetgum, was relatively consistent for the same insect species (Dowd PF and Lagrimini LM, unpublished).[1,5]

At present, most evidence suggests resistance is due to peroxidase-enhanced production of compounds toxic to the insects, as opposed to reducing nutritional quality or making tissues tougher (although these may be involved as well). When mortality occurs, it occurs at an interval shorter than that needed for caterpillars to starve to death when only water is provided.[1,5] If nutritional or structural effects were relatively more important than peroxidase production/detoxification of toxic metabolites for transgenic vs wild-type sweetgum, we would expect to see the same trend for *H zea* and *O nubilalis*.[6] The best evidence for the importance of a toxic effect is the detailed gravimetric nutritional studies performed with *L dispar* and sweetgum leaves.[6] In this instance, significant reductions of growth rates were noted, but these were not associated with significant effects on the insect's ability to digest or assimilate the leaves, suggesting that a toxic effect was most important.[6]

Although enhanced insect resistance by increasing peroxidase activity appears relatively consistent for the same insect species and tissues within the same plant genus[1,5] and family[1] (Dowd PF and Lagrimini LM, unpublished), enhanced resistance to insects through increased peroxidase activity can also potentially occur in distantly related dicotyledenous plants such as sweetgum.[6] Further study should yield answers to questions concerning appropriate peroxidase isozymes to express in suitable plant allelochemical environments for enhanced resistance to target insects.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standards of the products, and the use of names by USDA implies no approval of the products to the exclusion of others that may also be suitable.

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Natural pesticides and the evolution of food plants

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Abstract: Cyanogenesis, a naturally occurring pesticide, played an essential role in the origin of plant agriculture. When our ancestors were domesticating plants, they chose a disproportionate number of cyanogenic species.

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